Phytologia (August 2012) 94(2)

GEOGRAPHIC VARIATION IN LEAF ESSENTIAL OILS OF DOUGLAS FIR (*PSEUDOTSUGA MENZIESII*)

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ABSTRACT

The volatile leaf oils of Douglas fir (*Pseudotsuga menziesii*) were analyzed from throughout its range. The major differentiation found was the divergence of the inland populations (var. *glauca*) from the coastal and Sierra Nevada populations (var. *menziesii*). The oils of var. *menziesii* differed from var. *glauca* in their major components: camphene (0.4-17, 25-30%), β -pinene (25-38, 2.5-12%), sabinene (4-12, trace-0.5%), α -terpinene (2.1-3.3, 0.2-0.0%), γ -terpinene (3.4-5.5, 0.1-0.3%),

terpinolene (9.5-14.6, 1.2-1.6%), cis-p-menth-2-en-1-ol (0.7-0.8, 0.0trace), terpinen-4-ol (10-12.1, trace-0.5%), and bornyl acetate (0.2-1.3, 14.6-44.7%). The oil of the Sierra Nevada population (Sierra Nevada race of Snajberk and Zavarin, 1976) was quite similar to typical coastal Doug Fir from WA and OR and not as distinct as found in the oleoresin oils of Snajberk and Zavarin. Overall the oils of the inland variety (var. *glauca*) varied from Yellowstone southward with the AZ - NM oils forming a group along with the southern Mexico oils except for the population at Cerro Potosi that had a different oil. The Cerro Potosi oil differed from other inland (var. *glauca*) oils in having larger amounts of α -pinene, β -pinene, δ -3-carene and smaller amounts of camphene, limonene and bornyl acetate. The leaf oil of var. *oaxacana* did not differ from var. *glauca* from nearby populations in southern Mexico. The leaf essential oils of *P. macrocarpa* are also reported. *Phytologia 94(2):199-218 (August 1, 2012)*.

KEY WORDS: Pseudotsuga menziesii, var. menziesii, var. glauca, var. oaxacana, leaf essential oils, geographic variation, Douglas Fir.

Douglas Fir [Pseudotsuga menziesii (Mirb.) Franco] is a wide ranging, common forest tree in North America (Fig. 1). The history nomenclatural of the morass name is а (see http://www.plantsystematics.org/reveal/pbio/LnC/dougfir.html), but seems to be settled by James Reveal. In a recent treatment, Eckenwalder (p. 572, 2009) recognizes two varieties: var. menziesii and var. glauca (Mayr) Franco [cited as (Beissn.) Franco in Eckenwalder, 2009]. Eckenwalder (2009) did not recognize var. oaxacana Debreczy & Racz, described from Oaxaca (Debreczy and Racz, 1995).

The leaf essential oils of *P. menziesii* have been exhaustively studied by von Rudloff (von Rudloff, 1972, 1973, 1984, von Rudloff and Rehfeldt, 1980) who carefully documented the large differences in oils between coastal (var. *menziesii*) and inland (var. *glauca*) in many compounds including camphene (0.3-8, 20-30%), β -pinene (15-30, 5-10%), sabinene (2-12, 0.1-0.5%), α -terpinene 1-3, 0-0.3%), γ -terpinene (2-8, 0.1-1%), terpinolene (5-15, 0.5-3%), terpinen-4-ol (5-15, 0.5-3%), and bornyl acetate (0.5-5, 20-30%). However, von Rudloff's studies

were limited to the Pacific Northwest and northern US-Canada (south to Wyoming).

A second team from USDA, Forest Products, Richmond, CA (Snajberk and Zavarin), made extensive studies of the terpenoids from the oleoresin of Douglas Fir (Snajberk, Lee and Zavarin, 1974, Snajberk and Zavarin, 1976, Zavarin and Snajberk, 1973, 1975). In their most comprehensive study (Snajberk and Zavarin, 1976), they found four chemical races: coastal, northern inland, southern inland and Sierra Nevada. These are shown in Fig. 1, along with populations used in the present study. Notice the populations sampled in the present study are from each race: coastal (2), northern inland (1), Sierra Nevada (1), southern inland (2), plus Mexico (6) as well as *P. macrocarpa*. Douglas Fir in Mexico is principally found in small restricted populations, except for a larger area in Chihuahua in northern Mexico (Fig. 1). In spite of the very exhaustive studies of leaf and wood oils in the United States and Canada, nothing has been reported about variation in Douglas Fir oils in Mexico.

However, there has been work at the molecular level on Douglas Fir in Mexico. Li and Adams (1989) reported that allozymes divided the Douglas Fir into northern coastal (var. *menziesii*) and inland (var. *glauca*) with two subgroups (northern and southern inland). They did not find evidence of a subgroup of Sierra Nevada Douglas Fir as Snajberk and Zavarin (1976) found, based on the oleoresin oils. In addition, Li and Adams (1989) found a distinct pattern in the allozymes from population 103 at General Cepeda, Coah., MX and speculated that it might be *P. flahaultii* Flous (also recognized by Martinez, 1963). However, a nearby collection (104, La Encantada, near Zaragoza, NL) clustered closely with *P. menziesii* from New Mexico. So that case seems unresolved.

Gugger et al. (2010) examined mtDNA and cpDNA sequences and found support for coastal (var. *menziesii*) and inland (var. *glauca*) in the United States and Canada. No evidence was found for a Sierra Nevada taxon, but mtDNA suggested the inland (var. *glauca*) might be divided into northern and southern groups. In a subsequent study, Gugger et al. (2011) examined Douglas Fir from Mexico. They found considerable divergence in cpDNA from Cerro Potosi, NL and Jamé,



Figure 1. Distribution of *P. menziesii* with chemical races of Snajberk and Zavarin, 1976 and populations sampled in the present study.

Coah. from other Mexico populations. CpSSRs supported two clades in Mexico (Gugger, Fig. 4c), but that pattern was not supported in mtDNA (Gugger, Fig. 4a) or cpDNA (Gugger, Fig. 4b) data; in summary, they concluded that "Mexican populations were genetically distinct from USA and Canadian populations, but more closely related to the Rocky Mountain variety than the coastal variety". As Gugger et al. (2010) did not show data from Mexico, and Gugger et al. (2011) showed only data from that country, it is difficult to ascertain the relationship of Mexican populations to those of the USA.

Phenotypic analyses (Reyes Hernández et al. 2006) revealed that *Pseudotsuga* populations of northern Mexico are morphologically similar to *P. menziesii* var. *glauca* from southwestern USA, but the populations from central Mexico differed. They also found a population of NE Mexico (San Francisco) morphologically separated from the rest, even from those of the same geographical region, suggesting an effect of isolation. This population is just 15 km NW from the one from El Potosi analyzed here.

The purpose this paper is to report on geographic variation in the leaf essential oils of *Pseudotsuga menziesii* from the USA and Mexico.

MATERIALS AND METHODS

Plant material: P. menziesii var. menziesii (coastal/ Sierra Nevada): Adams 12918-12922, Olympic National Forest, Port Angeles, WA, 48° 02' 48.1" N, 123° 25' 04.08" W, 525 m; Adams 12745-12757, on serpentine, Oregon Mtn., OR, 41° 59' 59.1" N, 123° 47' 10.2" W, 895 m: Adams 12779-12783, 6 km e of Buck Meadows, CA, 21 km w of Yosemite NP on US 120, 37° 49.579' N, 119° 58.421' W, 1150 m. var. glauca: Adams 12556-12560, 13 km w of Cimarron, NM on US 64, 36.54684° N. 105.03321° W, 2125 m; Adams 12744-12748 (ex D. Thornburg, 1-5), 9 km ne of Pine, AZ on Hwy 87, 34° 27.422' N, 111° 24.115' W, 2250 m; Adams 12818-12822, 20 km e of Yellowstone NP, on US 14 at the Palisades, 44.45448° N, 109.78182° W, 1910 m; Adams 13056-13060, (ex M. Socorro González Elizondo 7777a-e), Cerro Potosi, NL, 24° 53' 9" N, 100° 13'14" W, 3141 m.; Adams 13061-13066, (ex Martha González Elizondo 4408-4409, 4413-4416) Los Altares, Dur., 25°2'56" N, 105°59'48" W, 2310 m; Adams 13082-13087 (ex Vargas-Hernandez J1-J6), El Chico Natl. Park, Mineral del Chico, Hgo., 20° 10' 16" N, 98° 43' 55" W, 2,765m, Nov. 4, 2011; Adams 13088-13094 (ex Vargas-Hernandez C1-C7), Cuatexmola,

Ixtacamaxtitlan, Puebla, 19° 30' 22" N, 97° 50' 21" W, 2,980m, Oct. 30, 2011; *Adams 13095-13100* (ex *Vargas-Hernandez* T1-T6), Ejido Paso National, Tlachichuca, Puebla, 19° 17' 47" N, 97° 19' 45" W, 3,000m, Sep. 30, 2011; var. *oaxacana: Adams 13101-13103, 13105-13106* (ex *Vargas-Hernandez 11-I6*, Paraje Peña Prieta, Oaxaca, 17° 09' 38" N, 96° 38' 07" W, 2,700m, Oct. 21, 2011. *P. macrocarpa: Adams 12776-12778*, USFS Eddy Arboretum, Placerville, CA. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU, CIIDIR and CHAPA).

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each of the taxa were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of the Adams Essential Oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Data Analysis - Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The leaf essential oils of *P. menziesii* var. *menziesii* (coastal) were found (Table 1) to be nearly identical to the reports of von

Rudloff (1972, 1973). This is remarkable considering the changes in gas chromatography from packed to fused capillary columns. The oils of var. menziesii differed (Table 1) from var. glauca in their major components: camphene (0.4-17, 25-30%), β-pinene (25-38, 2.5-12%), sabinene (4-12, trace-0.5%), a-terpinene (2.1-3.3, 0.2-0.0%), yterpinene (3.4-5.5, 0.1-0.3%), terpinolene (9.5-14.6, 1.2-1.6%), cis-pmenth-2-en-1-ol (0.7-0.8, 0.0-trace), terpinen-4-ol (10-12.1, trace-0.5%), and bornyl acetate (0.2-1.3, 14.6-44.7%). The oil from the Yosemite NP, Sierra Nevada (Yose in Table 1) population (cf. Sierra Nevada race of Snajberk and Zavarin, 1976) was quite similar to typical coastal Doug Fir from WA (ONF, Table 1) and not as distinct as found in the oleoresin oil of Snajberk and Zavarin. However. comparing coastal (ONF) and Sierra Nevada (Yose, Table 1) oils reveals the coastal oil is higher in sabinene (12.9, 4.0%), δ-3-carene (1.8, 0.5%), y-terpinene (5.4, 3.4%), terpinolene (14.6, 9.5%) and geranyl acetate (2.1, 0.9%), but lower in β-pinene (25.5, 38.0%), bornyl acetate (0.2, 1.3%) and citronellyl acetate (1.2, 2.8%). The inland group (var. glauca, Yell, AZ, NM, A Du, El C, Oax, Table 1) shows some differences between the northern inland (Yell) and southern inland oils (excluding Cerro Potosi) for camphene (17.0 vs. 24-30%), α-pinene (2.5 vs 3.2-9.1%) and bornyl acetate (44.7 vs. 16-32%).

The Cerro Potosi oil (C Po, Table 1) differed from other inland (var. *glauca*) oils in having larger amounts of α -pinene, β -pinene, δ -3-carene and smaller amounts of camphene, limonene and bornyl acetate. The leaf oil of var. *oaxacana* (Oax, Table 1) did not differ from var. *glauca* oils in nearby populations in southern Mexico.

Principal Coordinate (PCO) analysis of 24 terpenoids (boldface, Table 1) excluded one (Z- β -ocimene) whose maximum value was too small (0.4%). PCO of the resulting 23 terpenoids gave eigenroots that accounted for 48.7, 15.4, 7.9, 7.5 and 5.0% of the variation before the eigenroots asymptoted. The overall trends among the oils are seen in Fig. 2 where the major Principal Coordinate (PCO) accounted 49% of the variance among the populations. The first axis separated coastal (var. *menziesii*) and inland (var. *glauca*) populations. Notice that *Pseudotsuga macrocarpa* is well resolved (Fig. 2).

To examine variation among the *P. menziesii* populations, *P. macrocarpa* was removed form the data and a new PCO performed. PCO analysis of 24 terpenoids (boldface, Table 1) excluded Z- β -ocimene (max. value 0.4%) and humulene epoxide II (max. value 0.0). PCO of the resulting 22 terpenoids gave eigenroots that accounted for 57.6, 9.4, 8.4, 5.9 and 4.2% of the variation before the eigenroots asymptoted.



Figure 2. PCO of *P. menziesii* populations and *P. macrocarpa* based on 23 terpenes.

This resulted in an even greater amount of variance removed by PCO axis 1 (58%, Fig. 3). One can now see some differentiation among the inland populations. Note particularly the much lower similarity of Cerro Potosi oil to other inland populations (0.774), than seen between other inland populations (0.892, 0.888). There appears to be a slight north - south cline from Yellowstone - AZ, NM - Mexico populations (see tricyclene, α -pinene, camphene, β -pinene and bornyl acetate, Table 1).



Figure 3. PCO of 12 populations of *P. menziesii* using 22 terpenes. Dashed lines are minimum spanning links. Numbers next to the lines are similarities.

Another way to visualize the clustering and similarities is by use of a phenogram. Figure 4 shows the clustering of populations based on 22 terpenes. The coastal - inland split is the major trend.



Figure 4. Minimum spanning diagram based on 22 terpenes for populations of Douglas Fir.

One can also see that the oil from the Cerro Potosi population is the most unusual oil in the inland (var. *glauca*) group (Fig. 4). In addition, the divergence of the Yosemite NP (Sierra Nevada) population is clearly seen. The Mexico populations (excluding Cerro Potosi) are very uniform in their oils, including var. *oaxacana* (Oaxac. in Fig. 4). There is no support for the recognition of var. *oaxacana*, but it may be divergent in molecular characters.

To better understand the variation, geographic clustering was preformed. The resulting diagram (Fig. 5) clearly shows the uniformity of the oils in the inland group and the divergence of the oil of Cerro Potosi from other Mexico populations. Again, the oil of var. *oaxacana* from Oaxaca is shown to be very similar to nearby populations of var. *glauca*.

These oils data are similar to the cpDNA sequencing data of Gugger et al. (2011), showing Cerro Potosi population to be different from other Mexico populations. A recent study (Wei et al. 2011) found a similar pattern in mtDNA (coastal and inland groups), but they found the cpDNA of inland Mexico populations to differ from inland USA populations. Additional molecular studies (in progress) are needed to clarify the taxonomic relationship of Cerro Potosi and inland USA populations.

ACKNOWLEDGEMENTS

Thanks to Tonya Yanke for lab assistance and to Martha González and Abraham Torres for field assistance. This research was supported in part with funds from Baylor University.

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Figure 5. Contoured clustering (minimum spanning) based on equal interval clustering (0.07 similarity step intervals). Based on Fig. 4. data.

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Table Natio Rim, Pena appea	: 1. Comparison of leaf oil nal Forest, Yose = Yosen AZ, NM = Cimarron, NM Prieta, Oax., El C= El Cl r to separate the taxa and v	composi uite NP; - , C Po = nico Nat. vere used	titions fc var. <i>mei</i> Cerro P Cerro P K., H	or Pseu nziesii otosi,] lgo., O herical :	<i>dotsuga</i> var. <i>gla</i> NL, A E ax., Ma analyses	menzic uca: Y_i u = AI u = AI c = P.	<i>zsii</i> oils. ell = Y€ tares, D <i>macroc</i>	var. <i>m</i> ellowstc ur.; var <i>arpa</i> .	enziesii; me NP, . oaxacc Compou	ONF = ONF = AZ =] <i>AZ</i> =] <i>ma</i> : Oa unds in	= Olympic Mogolllon x = Paraje bold face
KI	cpd	ONF	Yose	Yell	AZ	MN	C Po	A Du	El C	Оах	Mac
884	santene	t		1.8	2.1	1.7	1.2	3.1	4.1	4.8	1
900	n-nonane	,	•	•		ı	t	•	•	ı	1
921	tricyclene	t	0.1	1.0	2.2	2.5	1.3	3.1	2.8	3.0	0.1
924	α-thujene	0.4	0.6	ı		•	0.1	•	•	,	,
932	a-pinene	6.4	7.9	7.0	9.7	4.5	18.0	13.5	10.6	11.6	14.0
946	camphene	0.4	1.3	17.0	24.6	26.4	13.8	30.3	30.0	28.2	1.0
969	sabinene	12.9	4.0	0.1	t	t	0.5	0.3	0.1	0.4	t
974	β-pinene	25.5	38.0	2.5	8.1	3.2	12.3	8.1	9.1	7.8	16.2
988	myrcene	1.6	1.8	1.9	1.5	2.0	7.8	3.0	7.0	1.6	9.2
998	n-octanal			t		ı	•	•	ı	ı	
1002	α-phellandrene	0.2	0.2	0.1	0.1	t	0.1	0.1	0.1	t	t
1008	ô-3-carene	1.8	0.5	0.6	0.7	0.8	3.9	1.9	0.1	0.3	0.2
1014	a-terpinene	3.3	2.1	•	t	t	0.2	0.1	t	t	t
1020	p-cymene	0.7	0.9	0.1	t	t	0.2	0.1	t	0.1	t
1024	limonene	1.6	2.0	6.6	5.0	6.0	2.8	6.0	6.0	5.4	20.6

							N T N		Curr	IVIAU
andrene	2.3	2.4	5.1	3.4	4.3	5.4	5.2	4.9	3.9	14.0
cimene	t		1	•	•	0.3	t	t	t	2.9
cimene	0.1	0.2	•	t	0.5	1.3	2.3	0.9	0.1	0.2
lene	5.4	3.4	0.1	0.1	0.2	0.3	0.2	0.1	0.1	0.1
nene hydrate	0.3	0.4	ı	•	•	ı	ı	•	ı	•
lene	14.6	9.5	1.2	1.0	1.2	1.6	1.5	1.2	1.3	0.6
henone, isomer	•	•	0.3	0.1	t	t	t	0.2	t	•
lbinene hydrate	0.2	t	ľ	•	•	'	1	•	•	ı
	0.2	1.7	1.3	1.5	3.2	t	0.7	0.4	•	0.4
Je	ı	t	1	•	'	1.2	•	•	•	•
lal	•	•	•	•	•	ţ	•	•	•	•
ienth-2-en-1-ol	0.8	0.7	·	t	t	t	·	•	•	•
nchol	t	0.1	t	0.1	t	t	t	t	•	t
holenal	0.5	•	0.1	•	•	0.1	١	•	t	0.1
octanoate	•	0.1	ı	t	'	•	•	ľ	•	
de	'	0.2	•	•	•	'	•	•	ı	•
nocarveol	١		1	'	1	t	ı	1	t	
-menth-2-en-1-ol	ı	0.4	•	t	t	ı	1	ı	ı	١

Mac	t	0.1	1	1	•	1	0.3	t	2.9	I	I	I	t	ı	1	1	1	0.2
Оах	0.3	0.1	•	ı	0.4	ı	t	t	0.2	ı	ı	ı	ı	ı	ı	ı	t	ı.
El C	0.5	0.6	ı	0.2	0.8	ı	0.2	t	0.3	ı	ı	ı	ı	ı	ı	,	0.5	ı
A Du	0.4	1.2	•	ľ	1.3	ı	0.3	t	0.5	ı	ı	ı	ı	ı	ı	ı	t	ı.
C Po	t	0.3	0.1	ı	0.4	t	0.4	t	0.4	•	t	ı	t	t	•	0.3	0.3	ı
NM	0.2	1.2	0.2	ı	1.5	ı	0.2	ı	0.8	t	ı	ı	ı	I		t	0.2	1
AZ	0.5	1.7	0.3	ı	2.2	ı	0.2	ı	0.2	t	ı	ı	ı	ı	ı	ı	1.5	ı
Yell	0.2	2.0	0.3	t	1.3	ı	0.5	t	0.3	0.1	ı	ı	ľ	ı	ı	0.2	0.3	ı.
Yose	t	0.1	0.4	ı	ı	ı	10.1	0.1	2.3	0.4	ı	ı	·	ı	ı	ı	0.9	ı.
ONF	t	t	0.5	t	ı		12.1	t	2.4	•		t		ı	t	•	0.2	
KI cpd	1141 camphor	1145 camphene hydrate	1148 citronellal	155 isoborneol	1165 borneol	1169 ethyl benzoate	[174 terpinen-4-ol	179 p-cymen-8-ol	1186 a-terpineol	190 methyl salicylate	195 methyl chavicol	196 cis-piperitol	201 n-decanal	204 verbenone	1207 trans-piperitol	218 endo-fenchyl acetate	1223 citronellol	1232 thymol, methyl ether

Mac	'	1	0.1	ı	•	t	1	1	0.2	t	t	1	6.1	1	'	0.6	0.2
Oax	t	1	25.2	1	1	•	1	ı	1	t	1	t	t	•	1	t	ı
EIC	•	•	20.7	•	·	ı	ľ	1	I	t	١	I	0.1	ı	1	ľ	ı
A Du	•	'	16.0	•	ı	ı	ľ	•	1	t	ľ	·	t	ı	•	ľ	•
C Po	0.1	1	14.6	•	0.1	0.1	•	0.3		0.2	'	0.4	0.1	t	0.1	0.2	0.5
MN	0.2	•	28.9	•	1	•	0.1	'	ľ	0.4	'	t	0.3	ľ	•	t	•
AZ	t	1	32.4	'	•	ı	0.1	1	1	0.1	1	ľ	•	•	'	t	•
Yell	١	•	44.7	•	ı	ı	0.3	1	ı	0.2		1	0.2	•	ł	ı	۰
Yose	t	t	1.3	t	ı	t	•	ı	•	2.8		ı	0.9	'		0.2	•
ONF	t	•	0.2	•	ı	•		I	ı	1.2	1	ľ	2.1	'	·	·	•
cpd	piperitone	neo-isopulegol acetate	bornyl acetate	trans-pinocarvyl acetate	tridecane	myrtenyl acetate	43,93,121,194	α-cubebene	α-longipinene	citronellyl acetate	neryl acetate	α-copaene	geranyl acetate	β-bourbonene	β-cubebene	longifolene	(E)-caryophyllene
KI	1249	1274	1287	1298	1300	1324	1342	1345	1350	1350	1359	1374	1379	1387	1387	1389	1417

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Mac		I	ı	0.8	ı	I	I	I	0.7	ı	ı	ı	ı	ı	I	0.3		
Оах	1	t	ı	ı	I	ı	ı	t	0.7	ı	I	0.2	ı	0.2	ı	0.5	ı	
EI C	. 1	ı	ı	ı	ı	1	ı	t	0.3	ı	ı	ı	ı	ı	ı	0.3	ı	
A Du	I	ı	ı	1	ı	ı	ı	t	0.1	ı	ı	0.1	ı	t	·	0.2	ı	
C Po	t	ı	0.2	0.1	t	t	0.4	0.1	0.4	0.4	0.4	0.5	ı	ī	1.0	1.5	0.2	
MN	I	ı	•	ı	ı	I	ı	t	0.3	ı	ı	t	ı	ı	ı	t	ı	
AZ	I	ı	ı	ı	ı	ı	ı	ı	t	ı	ı	ı	ı	ı	ı	ı	ı	
Yell	I	ı	I	ı	ı	ı	ı	ı	ı	ı	ı	ı	ı	ı	ı	t	·	
Yose	I	ı	ı	ı	·	ı	ı	ı	0.2	ı	ı	ı	ı	ı	ı	t	·	
ONF	ı	ı	ı	t	ı	ı	ı	ı	t	ı	ı	ı	t	ı	ı	t	ı	
cpd	β-copaene	trans-a-bergamotene	trans-muurola-3,5-diene	a-humulene	ethyl cinnamate	massoia lactone	trans-cadina-1(6).4-diene	γ-muurolene	germacrene D	trans-muurola-4,5-diene	epi-cubebol	a-muurolene	B-bisabolene	γ-cadinene	cubebol	ô-cadinene	trans-cadina-1,4-diene	
KI	1430	1432	1451	1452	1465	1471	1475	1478	1480	1493	1493	1500	1505	1513	1514	1522	1533	

Mac 0.2 0.8 0.2 0.2 0.8 0.7 0.6 Oax 0.3 0.1 0.1 0 EIC 0.2 0.1 0.1 A Du 0.2 0.1 0.1 C Po 0.6 0.2 0.2 0.2 0.2 ı ı MN 4. 2.0 AZ Yell Yose ONF humulene epoxide II caryophyllene oxide germacrene D- 4-ol (E)-α-bisabolene benzyl salicylate benzyl benzoate 119,107,91,202 epi-α-muurolol octadecadiene* pentadecanal* <u>43</u>,81,161,222 l-epi-cubenol epi-α-cadinol (E)-nerolidol hexadecanal 3-atlantone a-muurolol a-acorenol α-cadinol cpd 1616 1632 1814 1864 1574 1582 1608 1608 1627 1638 1638 l 644 1646 1652 759 887 1561 541 711 Z

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KI	cpd	ONF	Yose	Yell	AZ	MN	C Po	A Du	El C	Oax	Mac	
1889	heptadecatrienal*	ı	ı	ı	ı	I	0.1	I	t	I		
1937	cembrene	ı	ı	ı	t	t	0.1	•	•	•	0.2	
1943	iso-cembrene	ı	ı	ı	ı	t	•	•	ı	•	t	
1987	manoyl oxide	ı	ı	ı	ı	t	'		ı	1		
1992	ethyl hexadecanoate	ı	ı	ı	ı	ı	0.3		t	•	·	
2014	palustradiene	ı	ı	•	t	t	t	ı	ı	1		
2048	thunbergol	ı	ı	ī	t	t	0.1	ı	ı	ı	0.5	
2055	abietatriene	ı	ı	ı	ı	ı	t	ı	ı	ı		
2056	manool	ı	ı	t	t	t	1.0	,	•	t	t	
2087	abietadiene	ı	ı	ı	•	t	t	·	'	'		
2149	abienol	,	,	ŀ	ı	ı	t	•	•	•		
2165	9Z,12Z,15Z-octadeca-											
	trienoic acid, ethyl ester*	•	,	ı	ı	ı	0.2	•	•	•	•	
2300	tricosane(C23)	0.1	t	ı	t	t	'	,	ľ	•	0.3	
2313	abietal	•	•	•	•	t	ł	•	•	ľ		
KI = Unide	Kovats Index (linear) on antified components less th	DB-5 c an 0.5%	olumn. 6 are no	Compc ot repor	sitiona ted. Fo	l values or unkn	less tha	an 0.1% npound	ó are d s, four	enoted ions are	as traces (1 e listed, wi	h
UIC 10	The set for minering of the set o		ornnan									